QUANTITATIVE ESTIMATION

OF

URINE.

NEW SYSTEM OF RAPID ANALYSIS,

For Medical Men and Pharmacists.

ACIDITY, UREA, SUGAR, TOTAL URATES,
ALBUMEN, AND COLOUR.

By J. BARKER SMITH, L.R.C.P.Lond.

Author of "The Pharmaceutical Guide," &c.,
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Correct diagnosis demands routine examination of the urine in every first case, almost without exception. Care and observation will help us to convert every qualitative test into an approximate quantitative test, so that a mere qualitative test is rarely performed. Such a complete statement of the urine should be kept for future reference, and should accompany the introduction of a patient to a specialist, aurist, oculist, &c. It would spare the regular occurrence of experts missing general conditions of patients whilst treating their local troubles. Treatment necessitates, moreover, quantitative examination of urine to judge of the progress of the case.

We may, in a great measure, arrange our quantitative tests for urine in two main divisions.

I. Tests associated with the estimation of a sample of urine rendered *alkaline*. These are used to estimate *acidity*, *urea*, *sugar*, *total urates*, *phosphates*, *ammonia*, &c.

II. Tests associated with the estimation of a sample of normal or acid urine—*albumen*, *biliary salts*, *peptone*, &c.

General Procedure. (a)

Physical characters of the urine, such as the specific gravity and colour, precede the chemical examination. These are somewhat supplementary to the subsequent chemical characters. The normal or urochrome colour of urine, *i.e.*, yellowish brown, can be exactly recorded in numbers of which, by my scale, there are fifty recognisable between limpid and sherry-coloured urine. Twenty (*a*) centims of urine are placed in a small glass mortar furnished with a good lip, *carbonate* of soda solution, one in ten (Na_2 CO_3, $10H_2O$) is added to

(*a*) Centim is an abbreviation of cubic centimetre r gram volume.

neutrality or faint alkalinity, such quantity of soda solution records the acidity *in numbers*. The alkalinity being taken we add more carbonate of soda solution, in all four centims. The urea is then estimated *thermometrically* from one centim of such alkaline urine. Twenty centims of the alkaline urine are next measured, the remainder of the urine may be used to ascertain approximately the glucose percentage. The glass mortar being empty the twenty centims of alkaline urine are placed therein, one gram of ammonium chloride is added to the urine and stirred with the pestle until dissolved.

Five centims of such solution are then used to obtain the possible urate; the remainder is allowed to stand in the mortar for a quarter of an hour, when nearly the whole, ·03 gram per litre remains in the filtrate, of the urates are precipitated, the contents of the mortar are then poured upon a paper filter and the possible urate of the filtrate ascertained. The possible urate before and after precipitation affords *the absolute total urate by difference*. The two experiments to ascertain the possible urate do not occupy one minute. The urea estimation occupies about a minute. The glucose estimation is made in a few minutes. So that the whole procedure occupies less than an hour, *i.e.*, *acidity*, *urea*, *total urates*, *glucose*, *colour*.

There is one manipulative detail which must be ensured to secure accuracy, and this is most readily acquired by a few hours' practice. The acquirement of this manipulative skill is, for the medical man, the addition of a sixth sense, and one which gives him the means of ascertaining facts arising in all departments of medicine, rapidly, accurately and simply. The actual experiment never exceeds thirty seconds. Such

departure has allowed me in the case of several poisonous alkaloids—*morphia*, apomorphia, colchicine, veratrine, emetine—to co-ordinate in value *oxidation* and *emesis*. It has allowed me a masterly grip of albuminous food-stuffs, cereals, milk, bread-stuffs, &c. This departure from routine methods of applying permanganate as an oxidiser has enabled me to avoid many fallacies of analysis ; it is sanctioned by philosophical and chemical considerations as well as by results and convenience. The differences arising in oxidation by varying circumstances of *dilution*, *heat*, and *time*, made me recognise the *mutability* of chemical laws in the absence of rigorous definition of exact conditions. Hence I was enabled to give to a *regular method* the value of scientific precision and to establish the limits of error. I thus became possessed, in 1877, of another sense-equivalent and applied it to colours, urine, terpenes, chlorophyll, galenicals, &c. From such experiments arose biological facts of great interest whereby green plants in action by their chlorophyll stood co-ordinated with their white embryonic albumens in their *aëration potentiality*. Hence my new method for estimating urates in urine is not essentially different to the one introduced by me twenty years ago, nor very different to my method used at the same time for morphia in opium, quinine in cinchona, &c. *Vide Year-Book of Pharmacy* 1878. *My method of using acid permanganate has never varied.*

One gram of potassium permanganate is dissolved in 100 centims of distilled water. Such one per cent. solution may be kept unaltered.

One centim of the one per cent. solution is mixed with some water, about six centims of dilute sulphuric acid (about ten drops of the strong) added, and the whole is made up with pure water to 100

centims. Such a solution is alterable and must be made almost daily. Fifty centims of the acid permanganate constitute a *Norme*, used *invariably* for *recording*. Ten centims contain a *Subnorme*, used almost invariably for all *experiments*. It is also quite practicable to use a tenth norme of five centims.

Method.—Measure a subnorme into a two ounce flask. Fill a ten or five centim pipette with the solution to be oxidised. Take the flask between the finger and thumb of the left hand and the pipette in the right hand. Shake the flask gently and continuously whilst the solution from the pipette is delivered *regularly* in running drops nntil the last trace of pink shall have disappeared and the contents of the flask become as limpid as water. The experiment ought not to occupy more than thirty seconds, and should be practised until we are able to repeat experiments exactly, to a drop or two. Milk is a very convenient article to use for practice. Five centims of milk are diluted with water to twenty-five centims, the subnorme used; no calculation is required. We shall find 1·2 centims of milk to decolourise the norme, *i.e.*, the same quantity of the diluted milk the subnorme. One such experiment, moreover, allows us to instantly fix the albuminoid percentage of milk, for sugar and fat do not react. Again, we may proceed in the same way, first bringing up our subnorme to boiling point over a small spirit lamp; we shall now find that a smaller but regular quantity of oxidisable solutions will decolourise. The results of these two experiments expressed in centims, give us an *expression of oxidation capacity*, a mark of *integrity* for all oxidisable substances, *e.g.*, :—

EXPRESSIONS OF OXIDATION CAPACITY.

Cows' milk 1·2—·5	Woman's milk 3·3—·8
Goat's milk 1·5—·5	Asses' milk . . 2·5—·6

The above are most valuable *integrity* marks for all samples of milk ; the first terminal affords us albuminoid percentage, the second terminal gives total oxidisable solids. We may express all this in an abbreviation with the Roman figures to show dilution, *e.g.*, cow's milk, E O.C. 1·2—·5, V.

The norme will rarely require to be standardised if we use crystals of permanganate. I have found no variation of the crystals during twenty years, and I have kept the one per cent. solution (unopened) for ten years without alteration. However, we may most easily standardise the permanganate by solutions of ferrous sulphate or ferrocyanide of potassium. Such is given in a small brochûre, "Milk" by Author, (obtainable from Messrs. Baillière, Tindall & Cox). Just as we have an integrity mark for milk, which also admits of interpretation, so have we the same for urine, which refers to oxidisable substances *other than urea.*

Hence, without any chemical considerations whatever, we have a *constant* to use for the same sample of urine, which is most easily obtainable and whereby we may interpret many conditions of such urine.

So far is explained my *simple method* of using acid permanganate for nearly a thousand *oxidisables* in solution. Some of these are constituents of urine, notably the urates. Weigh a gram of uric acid and place it in a mortar, by means of twenty centims or sufficient liquor potassæ dissolve it in a litre of water. Of this 1 per thousand solution, 1·7 centim decolourises the subnorme, or 8·5 centims the norme. This standard allows us to ascertain the strength of any solution of urate in a few seconds accurately and simply. The constant dividend is 8·5, the divisor is the centims decolourising the norme, *i.e.*, the subnorme × 5, and the quotient is the *per thousand* of uric acid in solution. Urine contains other oxidisables in addition to the

urates ; referable to uric acid as a standard, we may say three or more times more oxidisables than urates. Salicylate of soda and other medicines are also to be reckoned with in such examinations. Suppose five centims of urine decolourise the norme, *i.e.*, really five centims of the urine diluted one in five decolourise the subnorme, we are able to conclude that the urine contains 1·7 per thousand of oxidisables reckoned as uric acid, of which a third or less is uric acid. Hence, acetate of lead and hydrochloric acid, were used by me twenty years ago (*d*) as precipitants of the uric acid, so that I obtained by means of the permanganate the percentage of total urates in urine by difference. I wanted still a perfect and rapid precipitant of urates for the acid permanganate to be an ideal quantitative test for total urates in urine. Such, I think, I have now approached in my alkaline ammonium chloride method recently published, and described in my former article. Nevertheless, we have a scientific and useful clinical test in the E.O.C. of a sample of urine, as important as any one character of the urine. Five centims of urine diluted with water to twenty-five centims ; the subnorme cold and the subnorme boiling afford us the two terminals without any calculation, *i.e.*, an exact character of the urine, the exact quantity of oxidisables reckoned as urates, *absolutely correct data to be interpreted according to our experience.*

EXAMPLES OF URINE (E.O.C.) V.

1. Diabetic urine ...	6·4	— ·5	Ratio twelve, abnormal.
2. Enlarged liver ...	2·2	— ·6	Ratio nearly four.
3. Normal urine ...	5·	— 1·	Ratio five.
4. Normal urine ..	3·3	— ·9	Ratio three and a half.
5. Debility	28·	— x·	No colour.
6. Acute rheumatism	4·6	— 1·4	Accession of relapse.

(*a*) *Vide* "Year Book of Pharmacy, 1878," and previously.

Saccharine and starchy matters react on the boiling subnorme and may be estimated by such means, urea does not react upon the acid permanganate cold nor boiling.

Characters of urates gleaned from practical work have clinical significance. Urate solutions are not stable, alkaline solutions soon deposit and weaker solutions quickly lose their strength, apparently absorbing air, hence uratic solutions may become more alkaline, if a portion be oxidised in respiration the alkaline condition of the blood might be increased. Unlike albuminous substances which have a three-fold decolourising power towards the boiling subnorme, urates react the same towards cold and boiling subnorme. Applying my alkaline ammonium chloride test to the blood, I have raised elsewhere new physiological points.

Oxidising and reducing agents, chlorinated soda and sulphocyanate solutions, may each produce oxalates from urates. Some solution of urate in a small thin phial corked, made to boil, and allowed to stand some months, produced some oxalates. A one per cent. chloride of sodium in a similar solution unboiled did not produce oxalates. Chronic rheumatism is often associated with oxalates. Fungologists are wrong in saying oxalates are not found in the hyphæ of fungi, I have found them cram the hyphæ of the common puff-ball, arising with the yellow colour. Experimentally, we may produce rheumatic symptoms in muscles and joints by uncooked mushrooms or by daily consumption of the coprini ; locally, some of the coagulants of albumen (trichloracetic acid solution 1-5) used too freely on the hands will produce similar pains over an extended area. Morphologically urates are allied sometimes to micrococci, have the same current movements, and are associated with schizomycetes.

Personally I recognise in *form* organised and unorganised *plasmodes* indistinguishable by the microscope.

The blood is always alkaline, this may not include all the tissues, aad we shall see that urates are *less* soluble in alkaline solutions, ammonia being present, than uric acid in aqueous solution. Two practical points in our daily work are forced upon our consideration. These are, the importance of ascertaining much more knowledge with respect to *ammoniacal* salts of urine and *acidity* of urine. Although I began with weaker solutions of ammonium chloride and finally selected 5 per cent. for the precipitation of urates, I have not marked down the comparative results; using my permanganate method any of my readers can fill up this gap. Some who use strong sulphuric acid and ammonium chloride in large quantities find some reaction from this source alone, *such fallacy does not pertain to my method.* This can be easily proved by adding a strong solution of ammonium chloride to a subnorme of acid permanganate. If in any case fallacy ttends my method, it will be found in an oxidisable substance precipitating with the urate or some substance present in the urine retarding the precipitation of the urate. In the case of acute rheumatism where the urates are high and salicylates are present we shall find it convenient to dilute the urine with an equal volume of water for greater accuracy.

Colour.– This belong to the physical characters of the urine. Here the weak acid permanganate is used to liberate iodine to match the colour of the urochrome.

Pour five centims of urine into a medium-sized test tube, into another test tube of the same size pour five centims of solution of potassium iodide (one per thousand). Pour the weak acid permanganate into the test tube, held with that containing the urine side by

side between the thumb and forefinger of the left hand, until the *colour* is the same in both test tubes.

The centims of acid permanganate used to produce this effect serve to record the colour of the urine. The maximum colour is *five*, as we note tenths we have fifty degrees of colour between *water* and *sherry* coloured solutions. This colour method is used by me to estimate *sugar* in the cereals ; the iodine solution formed by equal volumes of acid permanganate and *iodide* solution is my *alkaloid* and *albumen* indicator.

Special Procedure. (b)

1. *Acidity.*—Pour twenty centims of the urine into a small glass mortar, add drop by drop solution of *carbonate* of soda (1-10) until the urine is neutral, Let the centims or tenths of centims be noted and accepted as the *numerical acidity.* Such numbers viewed with statements of urea and sugar percentage are much more realisable than the acidity read as *oxalic, tartaric, acetic,* &c. A simple calculation converts such numerical acidity into other forms, such as acid phosphate of soda, &c., when required. I find an inch absorbent bandage, washed, dried, and soaked in tincture of litmus and again dried, a very good indicator, *a single thread is sufficient.* Suppose two centims of carb. soda solution have been used, *acidity is* 2.

Pour in more solution of carbonate of soda to full alkalinity, in all four centims, or more in very acid urines ; such is an alkaline urine turbid from earthy phosphates. It need not be filtered.

2. *Urea.*—Pour five centims of *good* chlorinated soda into a half-ounce phial, ascertain the temperature by means of a small thermometer. (*a*) Pour in one centim of the alkaline urine, cork the phial, hold it by the rim

(*a*) *Urea* Ammonia "*Sets,*" complete, with tables, obtainable from the wholesale—Maw—Dollond—and others.

so as to add no heat from the fingers, shake the contents *briskly* for ten seconds, *i.e.*, up and down twenty times or more, remove the cork, replace the thermometer, and in about *thirty seconds* read off the increase of temperature at its *acme* or rest. The increase of temperature multiplied by the factor ·28 will afford a product which is the percentage of *urea*. Add one-fifth total for dilution. The chlorinated soda solution may be proved good by pouring five centims of it into the phial, letting the thermometer remain *in situ* whilst *two grains* of powdered ammonium chloride are added, and the phial gently shaken. The temperature should mount up nearly twenty degrees for effective chlorinated soda solution.

3. *Sugar.*—Pour one centim of Fehling's solution into a test-tube and two centims of water, boil the contents, and whilst boiling, gently letting the alkaline urine in drop by drop from a pipette, a point is at last obtained when the *last trace of purple* has disappeared, and the supernatant liquid above the yellowish red precipitate is as limpid as water. The constant dividend is ·5, the divisor is the centims used, and the quotient is the percentage of sugar. Add one-fifth of total for dilution. If on being brought to boiling-point Fehling's solution become discoloured it is useless, and should a drop or two of the urine decolourise the copper solution such urine must be diluted so as to contain not more than one per cent. of sugar. I have used Fehling's solution as a quantitative test during twenty-five years, a white porcelain cup is not at all necessary. Formerly, I used ten centims of Fehling's solution undiluted, constant dividend five. A sheet of white paper, or a white plate under the flask assists the determination of *the last trace of purple*. I find that one centim in a test-tube, as directed, to possess many advantages.

Total Urates. — The acid permanganate method allows us to solve all problems associated with the numerous alkaline solvents of uric acid and urates. All the mineral waters for gout, all the remedies for the solution of urates can be tested *in vitreo* in a rapid and simple way. *Oxidisable substances* offer no difficulty as we can most easily standardise them. At all times a few minutes are sufficient to give us *expert* knowledge of all these subjects. It is here assumed that the reader has acquired the small amount of manipulative skill pertaining to my method. He is now in a position to abbreviate many of the urate tests, and to economise time and secure accuracy. Simple as the following method is for the estimation of urates, simpler than any other one test in the estimation of urine, it may in some cases be made a shorter and simpler test, or filtration may be spared. I mean by the use of the centrifuge, when even the acid permanganate may become unnecessary. *Vide* former articles.

4. *Total Urates.*—Pour 20 centims of the alkaline urine into a small glass mortar, add one gram of powdered ammonium chloride and dissolve. For a minute the solution is clear and we pour *five centims* into a measure and dilute it with water to 25 centims, adding a few drops of acetic acid to keep it clear. Ascertain the possible urate by means of the permanganate *subnorme*. Let the remaining 15 centims of urine stand in the mortar a quarter of an hour, filter through paper (return the first few centims to the filter) and measure off five centims of the *clear filtrate* and dilute it with water to 25 centims. Ascertain the possible urate in the filtrate by means of the permanganate *subnorme*. The *difference* of possible urate before and after filtration is the *absolute per thousand* of total urates, reckoned as uric acid, in the alkaline

urine. Add ·03 per thousand for the *trace* of urate in the filtrate, and *one-fourth* of total urate for the *dilution* by the alkaline ammonium.

In acute rheumatism during treatment by salicylates the urine may be found so that two centims or less decolour norme, in such a case the urine may be diluted with an equal volume of water at the start, for the sake of greater accuracy. These cases are immediately indicated by the first terminal of the expression of oxidation capacity. Sometimes it is convenient to use a strong solution of ammonium chloride (1—4), the precipitation is just as effective, but requires a somewhat longer time. We must remember in all cases to correct results according to the dilution.

To spare calculation I append herewith a table founded on the acceptance that eight-and-a-half milligrams of uric acid decolourise the norme (E.O.C. ·0085—·0085, M.).

Table of *possible urate* reckoned as *uric acid.*

Urine decolourising Norme. Centims.		Possible urate per thousand.	Urine decolourising Norme. Centims.		Possible urate per thousand.
2·0	equal	4·25	3·6	equal	2·36
2·1	„	4·04	3·7	„	2·29
2·2	„	3·86	3·8	„	2 23
2·3	„	3 69	3·9	„	2·17
2·4	„	3·54	4·0	„	2·12
2·5	„	3·40	4·1	„	2·07
2·6	„	3·26	4·2	„	2·02
2·7	„	3·14	4·3	„	1·97
2·8	„	3·03	4·4	„	1·93
2 9	„	2·93	4·5	„	1·88
3·0	„	2·83	4 6	„	1·84
3·1	„	2·74	4·7	„	1·80
3 2	„	2·65	4·8	„	1·77
3.3	„	2·57	4·9	„	1.73
3·4	„	2·50	5·0	„	1·70
3·5	„	2·42	5·1	„	1·66

(Table of *possible urates* reckoned as *uric acid.—Continued.*)

Urine decolourising Norme. Centims.		Possible urate per thousand.	Urine decolourising Norme. Centims.		Possible urate per thousand.
5·2	equal	1·63	6·8	equal	1·25
5·3	„	1·60	6·9	„	1·23
5·4	„	1·57	7·0	„	1·21
5·5	„	1·54	7·1	„	1·19
5·6	„	1·51	7·2	„	1·18
5 7	„	1·49	7·3	„	1·16
5·8	„	1·46	7·4	„	1·14
5·9	„	1·44	7·5	„	1·13
6·0	„	1·41	7·6	„	1·12
6·1	„	1·39	7·7	„	1·10
6·2	„	1·37	7·8	„	1·08
6·3	„	1·34	7·9	„	1·07
6·4	„	1·32	8·0	„	1·06
6·5	„	1·30	9·0	„	·94
6·6	„	1·28	10·0	„	·85
6·7	„	1·26	11·0	„	·77

5. *Albumens.*—All proteids in aqueous acid or alkaline solution are to be estimated accurately by the acid permanganate subnorme in less time than thirty seconds. Liquor potassæ is a general solvent of all albumens and will serve to make our standard solutions of the various albumens. No distinction is made in the four *albuminoids;* viz., *gluten*, *fibrine*, *serum albumen*, *casein*. Of a one per thousand solution of *dry albumen* or *albuminoid*, *i.e.*, the horn-like water free albumens, forty centims decolourise the norme or eight the subnorme. Hence the constant dividend is *forty* for the per thousand, or *four* for the percentage. The *divisor* is the centims of albumen solution decolourising the norme, and the quotient, respectively, is the per thousand or the percentage of dry albumen or albuminoids. *Vide* "Albumen," page 13 of brochure "Milk," article *one*.

Hence the first terminals of the *expressions of oxida-*

tion capacity given for milk in the first article are at once converted into percentage of albuminoids, viz., cow's milk, 3·3 per cent. of albuminoids ; woman's milk, 1·2 per cent. ; goat's milk, 2·7 per cent., &c. Workers who use my method for the estimation of albuminoids in flour and bread are accustomed to my term *soluble insoluble* albumen ; they recognise that a hundred centims of water retain in solution approximately four centigrammes of *coagulated albuminoids*. My permanganate method allows us to estimate solutions of albumen containing so small a quantity as a *grain* in a *pint*. So practice has evolved two methods for the quantitative estimation of albumen in urine, for the other oxidisables of urine prevent direct filtration of the albumen.

Method (*a*).—Coagulate by boiling and acidulating in the usual way. *Measure the opalescence.* For small quantities of albumen in urine. If trichloracetic acid be used in the cold (1 in 5) the opalescence is somewhat deeper.

Method (*b*).—Coagulate by boiling and acidulating in the usual way. Estimate the precipitated albumen by a factor and add a maximum for the soluble insoluble albumen. For large quantities of albumen.

The Opalescent Scale.

I devised the following opalescent scale for my own convenience, and published it only a few years ago. I have subsequently seen Dr. George Oliver's "Bedside Urine Testing." He has used alum and not sulphate of magnesia. Again, we should obtain double the amount of observable degrees of opalescence by using a half per cent. (·5 per 100) solution of caustic potash. However, the acid permanganate method will enable us to remodel the opalescent scale applied to the esti-

mation of albumen whenever it may be desirable. A two-cubic-centimetre pipette graduated in fifty markings, is everywhere obtainable for a shilling.

Five centims of two per cent. solution of sulphate of magnesia are poured into a *clean* test-tube, a drop or two of the one per cent. potash solution is added from the pipette to the point of *disturbance of limpid brilliancy;* the solution standing at 0 in the pipette, drop after drop of potash solution is then added to match the opalescence of the albuminous fluid. Range from one per twenty thousand of albumen in solution to one per two thousand or point of precipitation.

Formula.—The two decimal figures read as whole numbers, divided by two, give the centigrams of *dry* albumen or albuminoid per litre or albumen per thousand. If we use a half per cent. of caustic potash solution we shall have to divide by *four* instead of *two.*

The first column or pipette reading gives the *opalescent* scale for general use, the second column gives, in this case, the *albumen* per thousand.

It may also be used to measure ground mists arising from condensation over a cold or damp area, produced by wet soil, underground water, &c., *vide Invention*, Nov. 11th, 1893, page 990, and following articles, fifteen.

ALBUMEN SCALE FROM ·05 TO ·5 PER THOUSAND.

Pipette.	Albumen Per Thousand.	Pipette.	Albumen Per Thousand.	Pipette.	Albumen Per Thousand.
·10	·05	·24	·12	·42	·21
·11	·055	·26	·13	·44	·22
·12	·06	·28	·14	·46	·23
·13	·065	·30	·15	·48	·24
·14	·07	·32	·16	·50	·25
·16	·08	·34	·17	·52	·26
·18	·09	·36	·18	·54	·27
·20	·10	·38	·19	·56	·28
·22	·11	·40	·20	58	·29

Albumen Scale from ·05 to ·5 per thousand.—(*Continued.*)

Pipette	Albumen Per Thousand.	Pipette.	Albumen Per Thousand.	Pipette.	Albumen Per Thousand.
·60	·30	·74	·37	·88	·44
·62	·31	·76	·38	·90	·45
·64	·32	·78	·39	·92	·46
·66	·33	·80	·40	·94	·47
·68	·34	·82	·41	·96	·48
·70	·35	·84	·42	·98	·49
·72	·36	·86	·43	1·00	·50

The *formula* for albumen value deduced from the opalescence is so simple that we scarcely require the above table whether we use a half per cent. or one per cent. caustic potash solution.

My factor for converting albuminoid *volume per cent.* is derived from my experiments of flour albuminoids precipitated from weak alkaline solution by acetic acid.

In *twelve hours* there is no perceptible shrinkage, and *one gram of dry albuminoids* is contained in one hundred and sixty gram volumes of such albuminous *deposit.* Hence the factor for conversion is ·006 say for maximum shrunk albumen deposit, or ·0035 for deposit of an hour. Suppose a test tube contains an albuminous deposit after an hour which occupies one centim in ten centims of urine, it is ten per cent. coagulum by volume, or ·035 per cent. of dry albumen in the urine. We must add ·04 per cent. for the *soluble insoluble* albumen, *i.e.*, the urine contains ·075 per cent.

In flour analysis such precipitates may be re-dissolved in liquor potassæ and immediately estimated by the subnorme, for starch and sugar do not react on the acid permanganate, in urine we have to reckon with urates and other oxidisables.

As *centrifugal* sedimentation advances such deposits and precipitants will be examined with still greater care and accuracy. Many of the precipitants of albu-

men are oxidisable, *i.e.*, they react on the subnorme immediately. Such precipitants have for the most part been examined by me and their expressions of oxidation capacity given. *Tannic acid*, *carbolic acid*, *ferrocyanide* of *potassium*, &c. Trichloracetic acid and acetic acid do not affect the subnorme. Picric acid is also oxidisable. Taurocholate of soda solutions and peptone solutions mixed, produce an *opalescence*, both these are oxidisable substances. All peptone solutions are estimated as albumens by the subnorme; the E.O.C. of taurocholate of soda is about ·5—·15, C.

The opalescent scale applied to urates, phosphates, and biliary salts, will be further discussed in the next section.

The next section will contain some new and rapid methods which bear upon our subject, methods which have seemed to be of very great practical value and which may be used when necessary in place of others already described. Some of these are interesting in bearing directly upon a new method of urine analysis which is certain to come to the front, I mean the sedimentation of urine by a centrifuge. By means of the centrifuge we shall be able to estimate in a few minutes urates, phosphates, chlorides, and albumen.

Special Procedure. (c)

I. *The Yellowish-brown Colour Scale.*—This scale was exactly described and applied to normal urine as the urochrome colour scale, although the numerical colour was not converted into percentage of urochrome. Five centims of solution of potassium iodide (one per thousand) with five centims of the weak acid permanganate afford *fifty degrees* of colour; such scale can be extended by dilution. Both acid permanganate and chlorine liberate *iodine* from iodide solutions, so

chlorine solutions may be approximated by the colour scale. I have used the colour scale to estimate glucose and maltose, and the *iodine* solution, formed by mixing equal volumes of iodide solution and acid permanganate, is a valuable indicator of albumens, alkaloids, starch, erythrodextrin, &c.

Sugars do not react upon the cold subnorme, although their solutions may be estimated by the boiling subnorme. In order to estimate such solutions other than those of cane sugar, more easily, I devised a light caramelisation to bring them within reach of the cold subnorme. As such caramelisation is attended by a yellowish-brown colour, it is very easy to approximate weak glucose solutions by such colour alone, but it is not so exact a test as the acid permanganate applied after such caramelisation.

Potash treatment of saccharine solutions and their subsequent estimation by the acid permanganate cold. One centim of liquor potassæ is poured into a test tube and nine centims of the sugar solution (neutral). The contents are boiled *one minute* exactly from the first commencement of boiling. The colour scale indicates the percentage of sugar in the solution *by inspection.* Rule, divide degrees of colour scale by *ten.* Colour one is ·1 per cent., &c. The eye can detect the colour of the iodine liberated from the five centims of the water-clear iodide solution by a single drop or tenth centim of acid permanganate solution, and, theoretically, we can record ·01 per cent. glucose by its colour after "potash treatment," in practice the eye does not detect colour in solutions weaker than ·02 per cent. More potash or further boiling produce *more* colour, but such would be *altered* conditions of the test. The constant dividend for glucose

estimated by the permanganate *norme* is two and a half, the glucose solution must be allowed to cool after caramelisation. The constant dividend for maltose and lactose is *three* the centims decolourising norme give the divisor, and the quotient is the percentage. Those who are interested in cereals will find these subjects exhaustively treated by me in *The Miller*, 1892 to 1896—15 articles, "Flour and Bread." A sample of diabetic urine, diluted five times and subnorme used, gives *en rapport*, urine and norme, 6·4 centims before the "potash treatment," and ·55 centims after the treatment. The constant dividend for grape sugar is two and a half; now 6·4 = ·39 sugar and ·55 centims = 4·54 sugar, the difference is the real percentage, viz., 4·15 per cent. This sample of urine indicated by colour scale 5 per cent. of sugar, and by Fehling's solution 4·1 per cent.

The same colour scale can be applied sometimes to solutions containing traces of ammonia. Pour into a cylindrical twenty-five-centim measure, containing twenty-five centims of very weak solution of some ammoniacal salt, say one per ten million of ammonia, one centim of Nessler's solution, no discolouration is discernible; repeat the test with a stronger solution,one per million. There is now perceptible discolouration, one marked on our colour scale ·3. The first mark on the colour scale is ·1. Again, one per million is the ratio of the *tenth* milligram to the hundred centims or ·1 milligram, so that ·1 colour scale is equal to ·033 milligram *ammonia* per hundred centims. So that *proceeding in a regular way, as given above*, we ascertain the exact colour by our scale and divide by three, reading the quotient as milligrams of ammonia per hundred centims, or, further, multiply the quotient by

·7 to express the same in grains per gallon. In fact, the calculation is done by mental arithmetic. Perhaps a short table will make these statements clear :—

Ammon. Solution.	Colour after Nessler.	Ammonia in milligrams (fr ctions of) or per hundred thousand.
100 centims.	·1	·033
,,	·2	·066
,,	·3	099
,,	·4	·132
,,	·5	·165
,,	·6	·198
,,	.7	·231
,,	·8	·264
,,	·9	·297
,,	1 0	·333

2. *Urea as Ammonium Cyanate.*—I have introduced elsewhere the pipette method as an accurate one for ammoniacal salts and for urea solutions. Modern urea methods are essentially *ammonia group* methods and not simply urea tests. Let us take first my thermometric method already described. I find a centim of one per cent. solution of urea to give $3\frac{1}{2}^{\circ}$ of heat, a two per cent. solution to give 7°. Now, a centim of one per cent. solution of ammonium chloride gives 4° of heat, and a two per cent. 8°, a three per cent. 12°. The three per cent. is approximately *one* per cent. of *ammonia*. Hence, the heat *ratio* of urea and ammonium chloride is as seven to eight, or a solution of one per hundred and fourteen ammonium chloride is equivalent to a solution of one per cent. urea, *i.e.*, for standardising our thermometer, &c. Further, we have the factor ·08 (ammonia) for one degree of heat. Hence, in ammoniacal urine, total of *ammonia and urea* can be taken together in one minute by the thermometer test as ammonia, the latter being expelled by boiling with ten per cent. carbonate of soda, the urea can then be estimated as ammonia, and by a factor is at once con-

verted into *urea*, just as ammonia may be into an ammoniacal salt.

Urea *in my tests* functions as ammonium cyanate, *i.e.*, the ammonia radicle only is broken up. Hence one centim of one per cent. urea does not give me seven degrees, nor by my pipette 3·4 centims of gas, but only half. Those who would criticise these results should refer to my articles in *Invention*, Aug. and Sept. 1895. The factors for urea and ammoniacal salts are given (*a*) elsewhere.

Pipette Method.—It is practically the urea test already given performed in a five or ten centim pipette by simply inverting the *closed* tube, the fluid emitted is read as *nitrogen*. *It appears a most accurate method* for ammoniacal and urea solutions ; it is described in the articles just mentioned.

The principle is the same in my next method for the bed-side estimation of urea.

Bed-side Estimation of Urea.—It is very easy to estimate urea in urine at the bed-side by means of a half-ounce or one ounce phial. Two small file marks convert a one-ounce phial, graduated in teaspoonfuls, into a very effective *nitrometer* or ureameter for urea, ammoniacal solutions, peroxide of hydrogen, &c.

The half-ounce phial by a few similar marks, indicating 1·7 centims space between them at the top of the phial, becomes a very accurate ureameter. Pour water into the one-ounce phial, close with the thumb, and inverting the phial let out the water gradually to the level of the sixth mark from the now inverted bottom. Stand the phial down and file a mark below at the level of the water, pour out the water and file a vertical mark (*one*) at the level of the sixth mark. Such is a permanent ureameter. We pour *good*

(*a*) *Urea-Ammonia "Sets"—Dollond—Maw—and Wholesale.*

chlorinated soda solution to the first file mark, we add one centim of neutral or not very acid urine, closing the phial with the thumb, we shake briskly (for a minute) until the reaction is over, when we invert the phial and remove the thumb under the surface of a basin of water. We then read off the centims (quarter teaspoonfuls) of *escaped* fluid. Each centim is ·6 per cent. of urea. If there be much foam the phial may be allowed to stand and drain for a minute or two before reading off.

Urates by Opalescence and Precipitation.—A recipe for a copper precipitant of urates appeared in the *Journal de Pharmacie d'Anvers*, of May, 1896 ; and in the same journal for September, an important paper on Analysis of Urine, by Charles Pottiez.

I have compared the results obtained by the copper precipitant with those given by my alkaline ammonium chloride method, and I consider the copper test a valuable addition to our methods. For the sake of uniformity of procedure, I replaced alkaline hydrates first used in my test by *carbonate* of soda solution. Hence, *carbonate* of soda is used to render the urine alkaline as in my method, but the phosphates must be removed by filtration. The copper precipitant is added to ten or twenty centims of the *clear* alkaline urine so long as there is any flocculent precipitate produced. One centim of the precipitant throws down one milligram of uric acid. I find some difficulty in determining the *end reaction*, apart from filtration and re-application of the test to the filtrate ; this difficulty is due to the easily disturbed cloudy precipitate. Failing to read off the end reaction I have applied the solution to the estimation of urates by *opalescence* and by *precipitation*. As the hyposulphite of soda is a powerful deoxidiser I am *not* able to use the copper precipitant

with the acid permanganate instead of ammonium chloride. I believe that the copper solution will be useful for estimations by the centrifuge. All the chemicals used in the solution must be pure.

The Copper Precipitant.—Sulphate of copper, one gram and forty-eight centigrams ; crystals of Rochelle salt, forty grams ; hyposulphite of soda, twenty grams ; distilled water made up to a thousand grams.

A. Opalescence.—Pour five centims of the *clear* precipitant into a half-ounce phial, and one centim of the clear alkaline urine, mix. If no opalescence is produced there is probably less than ·2 per thousand uric acid in the urine, *i.e.*, total urates reckoned as uric acid. If equal volumes be used, even ·06 per thousand uric acid will disturb the brilliancy of the mixture. If the *one centim* of clear alkaline urine should produce an opalescence, such should be a measure of total urates, and referable to the opalescent scale. Experimentally, I am inclined to fix provisionally the rule—read off the terms of the opalescent scale, multiply by one and a half, and the quotient is the per thousand of uric acid. For example, the opalescence of a 1·2 per thousand of uric acid solution was found ·8. I am of opinion that an opalescent scale and the centrifuge will become in time very permanent and universal features in urine analysis. I am also of opinion that quantities used should be as uniform as possible ; the urea method and opalescent urate method serve as examples. So, in the estimation of bile salts using an acid urine, I should use five centims of *clear* peptone, and add one centim (*a*) of the urine and refer the opalescence to my scale. Such opalescence can instantly be converted into percentage of bile salts reckoned as taurocholate of soda.

The use of peptone solution for the quantitative

(*a*) If necessary, five centims.

estimation of bile salts is due to Dr. George Oliver. The limited value or uselessness of the Pettenkofer bile test, and other facts pertaining to the subject, are discussed in the fourth edition of Dr. George Oliver's book "Bedside Urine Testing." I must refer readers to that book for further information.

All solutions of peptones used in such tests can be immediately standardised by the acid permanganate. Suppose the *clear* solution of a one per cent. peptone solution be used, I may find by the permanganate that it is only ·4 per cent. The acetic acid added to the test solution would not affect the acid permanganate, but salicylic acid added to preserve the peptone is *oxidisable.*

B. Precipitation.—The copper precipitant gives a cloudy precipitate which deposits in ratio to the specific gravity. Hence, we are not able, except in some cases, to mix five centims of clear alkaline urine with five centims of precipitant, and letting them stand two or three hours read off the volume of the precipitate. Such will be possible when we use a centrifuge for sedimentation.

Procedure :—Mix five centims of the clear alkaline urine with five centims of precipitant (or more in samples containing more than one per thousand of uric acid). Let the mixture stand until it is flocculent, a few minutes, pour the whole on a small paper filter and collect the deposit. The *clear* filtrate is thrown away. Wash the deposit with water carefully *into* a mortar or glass, so as to obtain about ten centims, this is allowed to stand in a ten-centim measure *two hours.* The volume of deposit is then read off. Five milligrams of uric acid occupy the volume of two centims and three quarters (2·8 centims). Performed carefully I believe such experiments are exact. Sample of

urine :—Urine twenty centims, solution of carbonate of soda (1-10) five centims. Filtrate examined—Opalescence ·3 (half per cent. potash), *i.e.*, ·15 opalescent scale (one per cent. potash). Rule gives ·22 per thousand uric acid plus one fourth for dilution. Urine contains as indicated by *opalescence* ·281 per thousand.

Five centims of the urine gave by deposition, diffusion in ten centims of water, &c., ·8 centim of deposit Such reads ·28 per thousand to which we must add a quarter for dilution. Urine contains as indicated by *precipitation* ·35 per thousand uric acid. Such is a difference of ·07 per thousand.

A sample of urine from the same healthy man was examined after an interval of two days, by the alkaline ammonium chloride and acid permanganate method previously described.

7·2 decolourised before precip.	= 1·18	per thousand,	
8·4 „ after „	= 1·01	„	„
Difference	·17	„	„
Addition filtrate ·03, dilution ·05	·08	„	„
Total urates reckoned as uric acid	·25	„	„

The addition for trace of urate in filtrate is invariable, that for dilution variable.

A sample of urine from the same man was again examined after a similar interval, more in detail. E. O. C. 6·2—1·6, V. Colour ·6. S. g. 1011. Urea ·98 per cent.

7·9 decolourised before precip.	= 1·07	per thousand	
9·0 „ after „	= ·94	„	„
Difference	·13	„	„
Addition filtrate ·03, dilution ·04	= ·07	„	„
Total urates reckoned as uric acid	·20	„	„

The following results were obtained in a case of acute rheumatism (relapse). It serves to illustrate somewhat the effects of salicylate of soda. (Total urates reckoned as uric acid.)

Sept. 29th.—S. g. 1014. Colour 1. Urea 1·7 per cent. Total urates ·5 per thousand. Acidity 1·8. Salicylates recommenced.

Oct. 3rd.—S. g. 1030. Colour 5. Urea 3·3 per cent. Total urates 1·3 per thousand. Acidity 2·5.

4th.—S. g. 1027. Colour not taken. Urea 3·5 per cent. Total urates 1·6 per thousand. Acidity 3. Salicylates discontinued, bicarbonate of soda given.

5th.—S. g. 1017. Colour 1. Urea 1·8 per cent. Total urates ·67 per thousand. Acidity ·5.

6th.—S. g. 1020. Colour 1. Urea 2·6 per cent. Total urates ·93 per thousand. Acidity ·7.

7th.—S. g. 1020. Colour 1. Urea 2·3 per cent. Total urates ·77 per thousand. Acidity ·6.

8th.—S. g. 1021. Colour 1. Urea 2·3 per cent. Total urates ·84 per thousand. Acidity 1·2.

The estimation of the total urates in this case of rheumatism was made by the alkaline ammonium chloride method. The same method applied to blood, hair, patches of psoriasis, bronchial sputa, fluids of the (*a*) eye-ball, &c., for the microscope, led me to raise certain points elsewhere as regards the demonstration of urates in the blood by the microscope and their significance. The trace of uric acid left in the filtrate is only ·03 per thousand; such solutions by evaporation on a cover glass still evidence urates under the microscope. It would be interesting to know precisely the limits of size which urates may take, I am of opinion that urates of micrococcus form occur smaller

a) *Post-mortem* changes; compare similar changes in disease, from degeneration of tissues and *formation of ammonia*. *Vide* method.

than one micro-millimètre, also that amorphous urates, flocculent precipitates, &c., function as colloids in determining small plasmodic masses with uratic *sporules*. I believe if the work contained in such an article as "Report on the Morphology and Development of the Blood," by Dr. A. Edington, *British Medical Journal*, May 31st, 1890, could be repeated with urate stains and precipitants that there is much more to discover.

It appears to me that there are albocyte and microcyte forms of urates not yet differentiated by microscopists. We see sometimes an apparent albocyte of the blood lengthening into a hypha with endogenous sporules, one escaped and its place marked within the hypha, or, again, microcytes from the glands becoming red by exposure to light; very similar appearances may be noticed with urates and some of their precipitants.

I have found in the blood, skin, fauces, &c., of rheumatic patients minute plasmodes of urates, apparently, without preparation, and by treating hair with potash and the ammonium chloride method, again we have, morphologically at least, evidence of urates. I have been anxious that an expert microscopist should throw light on this subject.

Further, in some cases, the history of the urate seems sometimes indicated by the form of uric acid crystal determined by an acid, *i.e.*, it is only a question of time whether we use solution or solid ammonium chloride as precipitant in our test, but the whole morphology of the urate salts is different, so pathology.

Phosphates.—I published sixteen years ago a translation of Joulie's method of estimating phosphates. This author recommends the precipitation of phosphates before applying the uranium test. I think his

precipitant of phosphates will be found useful in the centrifugal method of estimating phosphates in urine.

Ammonio-magnesium Citrate (a) (Joulie).—In grams. Citric acid, 40; pure carb. magnesia, 2·2; distilled water, 20. Add to the *solution* ammonia. 40 (strong solution and water equal volumes). Make up with water to 100 centims. Use 10 centims for about 20 milligrams phosphoric anhydride.

As a simple precipitant—10 centims of urine, 3 centims of ammonio-magnesian citrate; make strongly alkaline, with about 2 centims of strong ammonia; shake the mixture, and allow it to stand in a graduated measure for an hour. Read off the precipitate as sodium phosphate, or divide it by five for phosphoric anhydride. Supplement, when necessary, by the uranium process.

Experiments as regards the toxic effects of the urine of infected persons, investigations of the constituents of such urine and that of digestive auto-intoxication, have enlarged the horizon of our daily diagnostic wants. At present such work is almost beyond our application in general medical practice. An enthusiastic friend has kindly placed an effective centrifuge, constructed by himself from very simple elements, at my disposal. Such work does not come within the scope of this short series of articles. "Special Procedure" (b) is written for the dispensing counter of the medical man, or for the pharmacist and dispenser. Its object is to get precise or exact quantation of *five* important constituents of urine with the least expenditure of time and money:—viz., *acidity*, *sugar*, *urea*, *total urates*, *albumen*, so as to secure a sort of physiological or pathological registration of patients with respect to urine as part of our daily

(a) The solution is acid.

routine practice. I am of opinion that pharmacists, who dispense our prescriptions at reduced fees when paid for by ourselves, should take up this simple definite branch of urine analysis at the uniform fee of half-a-crown. At present such could scarcely be done for a guinea, apart from the new and simple methods of this section. Such considerations do not mean depreciation of scientific work but its extension ; it means the elevation of medicine and pharmacy to their just status with respect to correct *diagnosis.*

Potentiality of Urine. Illustrative points :—

(a) *Phenols, cresols, pyrocatechin, hydroquinone,* &c., occur in auto-intoxication due to bacterial decompositions. Some of these principles, in other branches of technical work, have been submitted by me to the acid permanganate. Many are very powerful deoxidisers and in small quantities would help to make up that *terra incognita* of "possible urate" left after the precipitation of absolute total urate by the ammonium chloride method. Carbolic acid approaches uric acid in decolourising power, so hydroxylamine. Hydroquinone is more than three times as powerful as uric acid in decolourising power and is about as powerful as tannic acid. Pyrogallol, gallic acid, morphia, are all approximate in decolourising power and are twice as powerful as uric acid, the standard of apomorphine is as morphia.

(b) *Potentialities associated with albuminous principles.* Such are also very small factors in the "possible urate." Colloids are oxidisable substances in many cases, *e.g.*, gelatin and isinglass ; these substances will bear much consideration with respect to urates in the body. Pure, G. P. Swinborne's excellent products, gelatin and isinglass, have the same expression of oxidation capacity and, like other oxidis-

ables, their solutions can be immediately estimated by the acid permanganate. Pure gelatin and isinglass were found to be, E. O. C. ·23—·09, C. The first terminal informs us that *dry* gelatin approaches *moist* albumen or coagulated white of egg, corresponding to sixteen per cent. of *dry* albumen or albuminoids. The ratio is nearly that of an albumen. Solid mucous cast from intestine, hydrocele fluid, and semen, all approach in decolourising power, viz., 1·5—·2 ; such we see is less than three per cent. of albuminoids (4 ÷ 1·5). Ferrocyanide of potassium, the precipitant of albumen, is oxidisable, but less than dry albuminoid.

About six centigrams in one per cent. solution decolourise the norme, whilst four centigrams of albuminoid do the same. Dissolve one gram of muscular tissue, in shreds, in a few centims of liquor potassæ by allowing them to stand in a test tube, finally dilute with water to a hundred centims. Such is a standard of reference for all fluid meats, peptonised albumens, &c. Although we can refer such preparations also to their albuminoids per cent. Lean meat is approximate to coagulated white of egg ·25. Examples :—Condensed milk ·5 or eight per cent. of albuminoids, a popular meat extract is ·4, another is ·8, but meat extracts do not profess to be high in albuminoids. There is a fluid meat at a fabulous price which I value ; its first terminal is ·07 or fifty-seven per cent. of albuminoids ; diabetic or gluten bread will approach this high standard, &c. Suppose we precipitate such alkaline albumens, using thyroid and other glands, we have still to prove their organo-therapeutic value. Several chapters in " The Progress of Chemistry," by Dr. Thudichum, deal with allied subjects profoundly and comprehensively. I suspect, too, that urochrome is an oxidisable in ratio to other

oxidisables. As an ardent fungus eater and believer in polymorphism, I have for many years been interested in the close approximation of the lives of the larger and smaller fungi, especially in the changes or metamorphoses produced by larger fungi in wood, &c.

There is much to be done in this direction. Potentiality of urine is an almost unnoticed character of urine, and I can only take two general illustrative types—diastasic and peptonic actions. "*Taka diastase*," or fungus diastase, I have not yet submitted to my method. Here is a sample of diastase from the manufacturing chemist, E. O. C. 1·—·21, C. The first terminal indicates only 4 per cent. of albuminoids, the ratio is that of a malt extract rather than that of an albumen. Fehling's solution indicates 10 per cent. of maltose, the iodopermanganate shows starch and dextrin, functional power not ascertained. In testing functional power of diastasic preparations we use mucilages of potato starch, tous les mois, and arrow-root, never wheaten starch. Boiled potato is always satisfactory and good, if not maximum results, are obtained in a quarter of an hour. The amount of sugar formed measures functional value. Such diastasic action goes on, probably, in the manufacture of all green extracts; percentages of glucose reckoned on the extracts-reducing Fehling are—hemlock 22, henbane 18, belladonna 16, monkshood 16, &c. Uric acid in weak solution I found to have half the reducing effect of glucose on the copper solution, peptone the same as uric acid, albuminoid half that of peptone; these are simply results noted for my own guidance. The following experiments emphasise albumen potentiality and the direction of urine experimentation. Weigh a gram of finely ground wheat, place it in a small glass mortar with five centims of water, rub it diligently

with the pestle for two minutes (*a*), make the whole mixture up with water to twenty centims, filter through paper, examine the filtrate with a tenth norme, 5·8 centims decolourised or 58 the norme = 1·38 soluble albumen, or deducting the regular half per cent. of *soluble* insoluble albumen, ·8 per cent. of absolute soluble albumen. The glucose in this fine seed wheat is ·98 per cent.; there is also slight diastasic action. Repeat the experiment with a malted wheat flour (Triticumina), we find perhaps $2\frac{1}{2}$ per cent. of soluble albumen, or 2 per cent. after deduction, and about the same of glucose. Mark the potentiality of the soluble albumen, evidenced by two short infusions of a quarter of an hour.

Initial temperature 137°, glucose increased to 11 per cent., dextrin also increased. Initial temperature 170°, glucose increased to 18 per cent., dextrin also increased.

The peptone formed must be ascertained, not merely the albumen dissolved. As regards peptonising ferments, the acid permanganate allows us to follow the details minute by minute in the simplest possible way, even in an oxidisable menstruum, apart from other changes. Glycerin and alcohol do not react upon the cold acid permanganate, both react upon the boiling subnorme and approximate in their decolourising power E. O. C. 0—·6, C. Taurocholate of soda and scale pancreatin approximate in oxidation capacity (·5—·15). Salicylate of soda ·05—·005, salicylic acid about the same. Dried mucus experimentally corresponds to 33 per cent. of albuminoid equivalent. Pepsina porci was found ·25—·06, C., as white of egg, or as we see by first terminal 16 per cent. albuminoid. Essence of pepsin. Two centims decolourise subnorme or 10 centims the norme, *i.e.*, two and a half per cent. pepsin or ·4 per cent. of

(*a*) For total albuminoids add four centims of liquor potassæ.

possible albuminoid. Oxalates approach uric acid in decolourising power, but they do not react immediately on the cold norme. Resinous substances, terpenes and colours, are all very oxidisable substances and in some cases may swell the "possible urate." The following substances have been examined by me and the results published in various technical journals. Chlorophyll, green extracts, aromatic waters and essences, nux vomica, hop, oxidisable alkaloids, arsenites, sulphites, wool, silk, cereals, &c. All oxidisable substances may be estimated in the same simple way. Dissolve oxidisable substances in water when soluble, or aid the solution by liquor potassæ or by an acid. My method of oxidimetry will allow us to obtain in a few minutes, or in a few seconds in most cases of liquids and solutions, *primâ facie* evidence of *integrity* for nearly all the preparations of the pharmacopœia. Hence, the weak acid permanganate can ever be at hand in the surgery or in the pharmacy. Made of uniform acidity we can always use such for its acid value, the permanganate being in most cases an unimportant quantity. The subnorme contains ten minims of dilute sulphuric acid, and when properly diluted is one dose.

Globulin and mucin may occasion errors in the albumen test. The urine is filtered; one part of the *clear* filtrate diluted with twenty volumes of water and acidulated with a drop of acetic acid, globulin determines a turbidity, globulin is mostly associated with albuminuria. Or to the filtered urine, undiluted, acetic acid is added, when mucin determines layers or deposit insoluble in excess of the precipitant. So we add to mucous urine some acetic acid and after a time filter and test the filtrate for albumen. Purdy says that the ferrocyanic test is "a most trustworthy test," it

must be applied exactly as follows :—Pour into a clean test tube one or two centims of acetic acid, and two or three times that amount of solution of ferrocyanide of potassium (1—20), mix well, add the urine to the depth of two-thirds, any cloud or precipitate *is albumen.* Applied in this way the mucin reaction is avoided, whilst all forms of albumen are precipitated. Forms of vegetable mucus, starch, and gum, react only on the boiling subnorme, these two substances are 0—·3, C. Sulphocyanides occur in urine, sulphocyanide of potash is four times stronger than uric acid in decolourising power, they form an insignificant factor of the "possible urate." Sulphocyanides and cyanides may be estimated by both my methods of oxidimetry. Blood serum with its colour gave E O. C. ·23—·075 C., clot, much stronger. Blood in water can be estimated in the usual time of a few seconds by means of the acid permanganate.